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論 文 題 目　　　　　Exploration of the functional roles of polyamine oxidases in *Oryza sativa* and *Arabidopsis thaliana*（イネ及びシロイヌナズナにおけるポリアミン酸化酵素の機能的役割の解明)

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論文内容の要旨

Exploration of the functional roles of polyamine oxidases in *Oryza sativa* and *Arabidopsis thaliana*
(イネ及びシロイヌナズナにおけるポリアミン酸化酵素の機能的役割の解明)

分子応答制御分野
KIM DONG WOOK

Plant major polyamines (PAs) are diamine putrescine (Put), triamine spermidine (Spd) and two tetraamines, spermine (Spm) and thermospermine (T-Spm) (Fig. 1). These biogenic amines play important roles in various physiological processes including growth, development, senescence and adaptive responses against environmental changes. PA contents are mainly controlled by equilibrium between biosynthesis and catabolism. PA biosynthetic pathway is well established in plants (Fig. 2). Put is synthesized from either ornithine or arginine by ornithine decarboxylase (ODC) and arginine decarboxylase (ADC), respectively. In Arabidopsis, ODC pathway is missing. Put is converted to Spd by Spd synthase (SPDS). Spd is further converted to Spm or T-Spm with the reactions catalyzed by Spm synthase (SPMS) and T-Spm synthase (ACL5 or TSPMS), respectively. SPDS, SPMS and ACL5 reactions require another substrate, decarboxylated S-adenosylmethionine (dcSAM), which is derived from S-adenosylmethionine (SAM) by the reaction of SAM decarboxylase (SAMDC). On the other hand, knowledge on PA catabolic pathway is incomplete. Two enzymes, copper-containing amine oxidase (CuAO) and flavin-adenine dinucleotide associated polyamine oxidase (PAO), are involved in the pathway. Arabidopsis contains at least 10 CuAO genes and 4 out of them were partially characterized. In general it is believed that CuAO can catabolize diamine Put and generate 4-aminobutanal, ammonia and H_2O_2 (Fig. 3). Maize ZmPAO is the most studied plant PAO. This enzyme catalyzes a reaction, the so-called terminal catabolism. Spd and Spm are oxidized and converted to 4-aminobutanal and N-(3-aminopropyl)-4-aminobutanal, along with 1,3-diaminopropane and H_2O_2 (Fig. 3). Barley HvPAO1 and HvPAO2 also showed the activity to catalyze the terminal catabolism. However, the recent research on Arabidopsis PAOs revealed that plant has the PAO catalyzing alternative reaction called 'back-conversion'. This type of PAO interconverts Spm and T-Spm to Spd and/or further to Put, along with 3-aminopropanal and H_2O_2 production (Fig. 3).
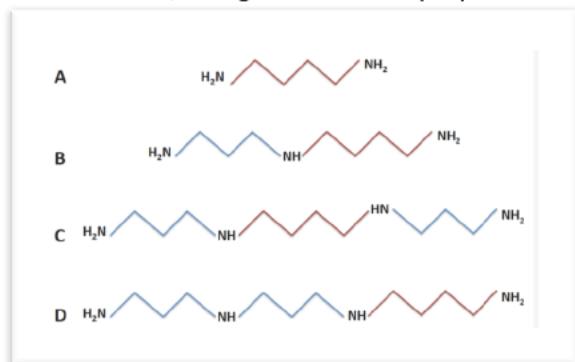

Figure.1. Structure of major polyamines in plant.
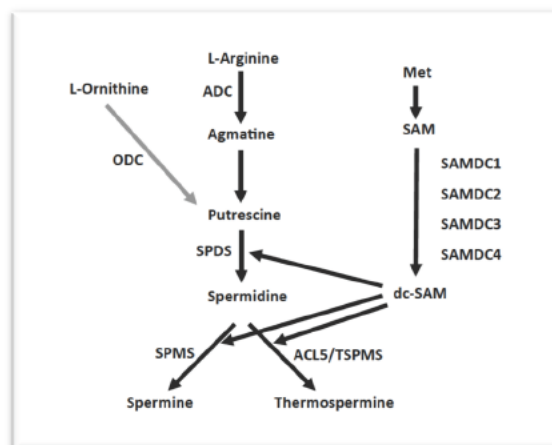A Putrescine; B Spermidine; C Spermine;
D Thermospermine.

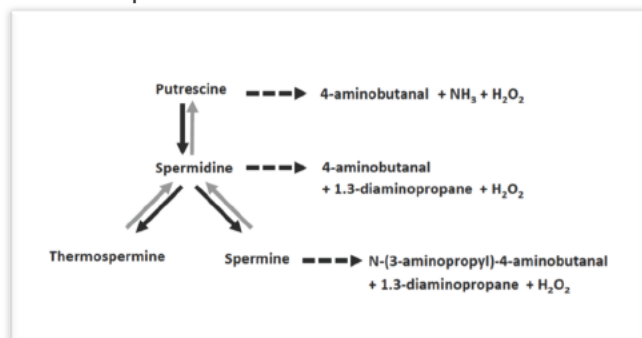**Figure2**. Polyamine biosynthesis pathway in plant.

Figure. 3. Polyamine catabolism in plant. Terminal catabolism- and back conversion- pathways are indicated by black arrow and gray arrow, respectively.

With the above background, I have studied several PAOs in *Oryza sativa* and *Arabidopsis thaliana*. The *O. sativa* genome contains 7 *PAO* isoforms which are termed *OsPAO1* to *OsPAO7*.

In chaper 1, I have characterized the expression of 7 *OsPAO*s and found that *OsPAO3*, *OsPAO4* and *OsPAO5* are abundantly expressed in young seedling stage and in maturing stage of flowers while *OsPAO1*, *OsPAO2*, *OsPAO6* and *OsPAO7* were expressed at very low levels with different tissue specificities. Then, the recombinant OsPAO3, OsPAO4 and OsPAO5 proteins were purified and characterized. OsPAO3 favored Spd as substrate followed by T-Spm and Spm. OsPAO4 and OsPAO5 shared similar PA substrate specificity. Both the enzymes preferred Spm and T-Spm but not Spd. The analyses of the reaction products revealed that those three OsPAOs catalyze the back conversion reaction. Furthermore, the amino acid sequences of their carboxy termini are SRL (OsPAO3 and OsPAO5) and CRT (OsPAO4), respectively, indicative of the peroxisomal targeting signal. In fact, they localize in peroxisomes in plant cells.

Taken together, I conclude that *OsPAO3*, *OsPAO4* and *OsPAO5* are constitutively and highly expressed in *O. sativa* and their products localize in peroxisomes and catalyze PA back conversion reaction.

In *Arabidopsis thaliana*, there are five *PAO* genes, *AtPAO1* to *AtPAO5*, and all their products, except AtPAO5, have been biochemically characterized. Thus, in chapter 2, I examined *AtPAO5* and its gene product AtPAO5. The recombinant AtPAO5 showed different pH optima for Spm (pH 7.5) and for T-Spm (pH 6.5). Under both pH conditions, Spm and T-Spm were converted into Spd by AtPAO5 but not further to Put. The result indicates that AtPAO5 is also a back conversion enzyme. I further addressed the physiological function of AtPAO5. For this purpose, I used the loss-of-function *pao5* mutants. Two allelic mutants, *pao5-1* and *pao5-2*, specifically contained about two-fold higher T-Spm levels compared to wild type (WT) plants. Those mutant plants have shorter inflorescence stems and smaller and distorted rosette leaves compared to WT plants (Fig. 4). Although WT and *pao5* mutants at the seedling stage were indistinguishable, low T-Spm doses could arrest the growth and development of the *pao5* mutant aerial parts in a T-Spm-specific manner. Introduction of the wild type *AtPAO5* gene into the *pao5* mutant resulted in growth recovery and a concomitant decrease in T-Spm content, demonstrating that AtPAO5 is essential for T-Spm oxidation.

The *pao5* mutant, lacking T-Spm oxidation, and the *acl5* mutant, lacking T-Spm synthesis, both showed severe growth defects, confirming the critical role of T-Spm in plant growth and development (Fig. 5).
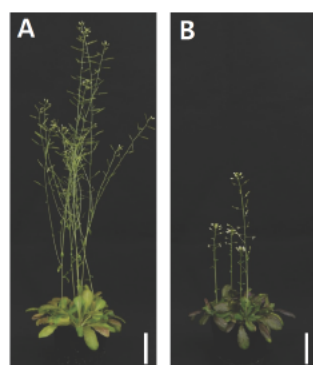

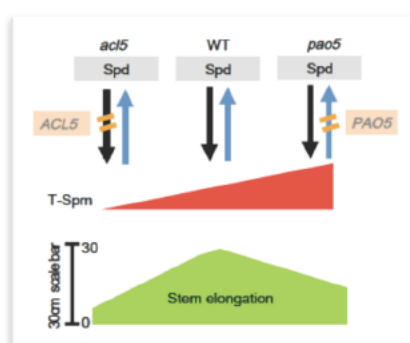Figure 4. Growth phenotypes of (A) WT (Col-0) and (B) *pao5-2*.

Figure. 5. Model describing the importance of T-Spm metabolism in Arabidopsis. In WT, both T-Spm synthesis, catalyzed by ACL5, and T-Spm back-conversion, catalyzed by AtPAO5, are functional, so plant grows well. In *acl5*, which cannot synthesize T-Spm, stem growth is severely disrupted (Hanzawa et al., 2000). The *pao5* mutant, which lacks T-Spm catabolism, similarly shows stem growth retardation and changes in leaf number and morphology.

Publication list
1. Yusuke Ono*, Dong Wook Kim*, et al. (2012) Amino Acids, 2012, 42: 867-876 (*equal contribution)
2. Dong Wook Kim et al. Polyamine oxidase 5 is essential for thermospermine catabolism and Arabidopsis growth. In preparation

論文審査結果の要旨

　金東煜は、イネおよびシロイヌナズナのポリアミン酸化酵素(polyamine oxidase, *PAO* と略)遺伝子に関する研究を行った。イネには 7 種の PAO 遺伝子(*OsPAO1〜OsPAO7* と命名)が存在する。彼は、イネの幼植物期と開花期の２つの異なる生育ステージで *OsPAO3*、*OsPAO4* そして *OsPAO5* が共にほぼ全ての組織で高発現していることを明らかにした。これらの cDNA を単離し大腸菌の系にて精製した組換え体酵素は、いずれもポリアミンに対する基質特異性は異なるものの生合成の逆反応である逆変換型の反応を触媒すること、を明らかにした。またこれら3種の酵素は、植物細胞内のパーオキシゾームに局在することも明らかにした。この結果は，単子葉植物であるイネに従来トウモロコシや大麦で見出された末端分解型のPAOとは異なる反応様式である逆変換型PAOが存在することを示した最初の報告となった。

　次に、彼はシロイヌナズナに存在する 5 種の *PAO* 遺伝子の内、唯一特徴づけがなされていなかった *AtPAO5* 遺伝子および組換え AtPAO5 酵素の特徴づけ、さらには植物体内での *AtPAO5* 遺伝子の生理的機能を解析した。大腸菌から精製した AtPAO5 組換え酵素は、スペルミンおよびサーモスペルミンをスペルミジンに逆変換する酵素であることを示した。また *AtPAO5* 遺伝子が機能を失った変異体シロイヌナズナ植物 *Atpao5* は、サーモスペルミン含量が野生型植物の 2 倍となるが、他のポリアミン含量に変化がないことを示した。さらに、通常の栄養寒天培地上の両者間の生育に差は見られないが、培地に低濃度のサーモスペルミンを添加すると *Atpao5* の地上部の成長が著しく阻害されること、この現象はサーモスペルミン特異的に起こることを示した。*Atpao5* 植物に正常な *AtPAO5* 遺伝子を導入すると、サーモスペルミン添加培地での生育阻害が回復することも示した。以上から、AtPAO5 はサーモスペルミン酸化酵素であると結論した。*Atpao5* 植物と野生型植物を土壌に生育させると生育後期において変異型植物の茎の伸長が有意に阻害されることも見出した。従って、シロイヌナズナにおいてサーモスペルミン量は厳密に調節される必要があることが考察された。

　上記の内容は、金東煜が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，金東煜提出の論文は，博士（生命科学）の博士論文として合格と認める。